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(54) Title: COMPOUNDS AND METHODS FOR THE INHIBITION OF PROTEIN-PROTEIN INTERACTIONS (57) Abstract Compounds having molecular weights of less than 1500 daltons that non-covalently interact with target proteins and covalently bond to target proteins at amino acid side chains that are not part of enzyme active sites, in which covalently bound portions of the compounds sterically block the binding of the target proteins to other proteins, are disclosed. The compounds react with forward reaction rates at least 100 times faster than the forward reaction rates at which the compounds bond to the side chains of the corresponding free amino acids under physiological conditions. Compositions including these compounds and methods for preparing these compounds are also disclosed.		

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COMPOUNDS AND METHODS FOR THE INHIBITION OF PROTEIN-PROTEIN INTERACTIONS

Background of the Invention

The invention relates to compounds, compositions, and methods for blocking protein-protein interactions.

Many pharmaceutical drugs act by blocking the binding of an enzyme to its substrate. In order for these drugs to block this interaction effectively, they must bind to the target enzymes more tightly than the substrate binds. Binding strengths are determined in part by the number of favorable contacts between the compounds. Since most enzyme substrates are small molecules, small molecule drugs can be engineered to make as many (or, if desired, more) contacts with the enzyme than does the substrate, facilitating tight enzyme interactions. As an illustration, Fig. 1A shows an enzyme 1 bound to its substrate 2. Fig. 1B shows the enzyme 1 bound to a small molecule drug 3. In addition, because enzymes work by lowering the energy of the transition state between substrate and product, the enzyme binds to especially tightly to transition state analogues. Drugs that resemble the transition state can bind more tightly to an enzyme than does the normal substrate, again affording an opportunity for antagonist-type drug design.

In contrast, it is generally more difficult to engineer small molecule compounds that block the interaction of a target protein with another protein, because interacting proteins usually contact each other over large surface areas and make many favorable contacts. It is therefore difficult for a small molecule to have a greater number of favorable contacts with a protein than does another protein. As an illustration, Fig. 1C shows two such interacting proteins, 4 and 5, and indicates the large number of favorable contacts between these proteins.

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In addition, interacting proteins do not possess transition states analogous to those exhibited by substrates and their products. Thus, there is no specialized conformation that a drug can mimic to bind more effectively to a target protein.

There are many instances in which it is therapeutically useful to
5 block the interaction of a target protein with another protein. Examples of biological events that involve protein-protein interactions include signal transduction, transcription, protein ligand-receptor interactions, and protein assembly.

The ability to block these processes specifically facilitates the
10 development of therapies for diseases that are currently difficult to treat. Accordingly, compounds that block interactions such as those described above represent potentially useful drugs for treating, preventing, or reducing the severity of certain diseases or their symptoms. For example, viral infections (such as herpes, hepatitis C, HIV, and influenza infections) could be treated
15 with compounds that block the assembly of viral proteins, or with compounds that prevent the ligand-receptor interaction of a virus attaching to a host cell.

Summary of the Invention

The invention features a compound having a molecular weight of less than 1500 daltons that non-covalently interacts with, and covalently bonds
20 to, a target protein at an amino acid side chain that is not part of an enzyme active site; a covalently bound portion of the compound sterically blocks the binding of the target protein to a second protein. The compound bonds to the target protein with a forward reaction rate that is at least 100 times faster than the forward reaction rate at which the compound bonds to the side chain of the
25 corresponding free amino acid under physiological conditions. The compound may bond in such a way that essentially all, most, or only a small portion, of

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the compound remains covalently attached to the target protein; another portion of the compound may serve as a leaving group. For example, in some instances, only an acyl group, preferably an acetyl group, remains attached to the target protein.

5 A preferred compound bonds to the target protein with a forward reaction rate that is at least 1000 times faster than the forward reaction rate at which the compound bonds to the side chain of the free amino acid under physiological conditions, and more preferably bonds with a rate 10,000 times, or 100,000 times faster. The compound is preferably a synthetic compound.

10 In addition, a preferred compound has a covalent bonding rate constant with the side chain of the free amino acid of less than 10^{-5} /M/sec at room temperature under physiological conditions, and more preferably has a rate constant of less than 10^{-6} /M/sec, or 10^{-7} /M/sec at room temperature under physiological conditions.

15 The non-specific covalent bonding rate constant for penicillin to form stable bonds with amino acid side chains, such as those of serine and lysine, is about 8×10^{-6} /M/sec. Because penicillin is a useful drug whose side effects, which are results of its reactivity, are considered to be acceptable, it is expected that the side effects resulting from the non-specific reactivity of drugs
20 with similar or smaller covalent bonding rate constants will also be acceptable.

 A preferred compound also includes a Specificity Group whose removal results in the bonding reaction rate with the side chain of the corresponding free amino acid being substantially unchanged, and the bonding reaction rate with the target protein being reduced to a rate that is substantially
25 similar to the bonding reaction rate with the side chain of the free amino acid; the compound also includes a Bonding Group that forms the covalent bond with the target protein. In this compound, modification of the Specificity

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Group does not substantially alter the bonding reactivity of the Bonding Group. In such preferred compounds, the Bonding Group and Specificity Group are connected by appropriate linkers, so that, for example, electronic effects are not transmitted from the Specificity Group to the Bonding Group. An example of
5 an appropriate linker is an alkyl chain having 2-12 carbon atoms.

Preferred target proteins include kinases, viral coat proteins, STAT proteins, oncogenes, transcription factors, and extracellular protein ligands, protein domains, and their receptors. More specifically, preferred target proteins include MCP-1, Fos, and IL-1 beta. In a preferred compound, the
10 Bonding Group forms a covalent bond with the side chain of the amino acid of the target protein as a result of the intrinsic reactivity of the Bonding Group.

The purpose of the Specificity Group is to direct the compound to a particular protein target and to position the Bonding Group near an amino acid side chain on the target protein; the Bonding Group will therefore have a high
15 effective concentration, relative to the amino acid side chain, and will react with the side chain. An initial Specificity Group may be obtained by screening conventional chemical compound libraries for compounds that non-covalently interact with the target protein. Alternatively, the initial Specificity Group could be obtained by rational drug design, or by using a peptide that is known
20 to bind to the target protein.

For comparison purposes, the side chain of the free amino acid that corresponds to the amino acid that forms a covalent bond to the B group is used, rather than a side chain on a non-target protein. The side chain of the corresponding free amino acid can always be clearly defined; in addition, it will
25 not be subject to environmental influences, such as steric factors, that may vary from one non-target protein to the next.

It is useful, during the improvement of Bifunctional Blockers that is

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described in detail below, for the Bonding Group and Specificity Group to be connected so that modification of one Group has little effect on the other. In brief, improvement of Bifunctional Blockers is accomplished by systematically improving the Specificity Group and weakening the reactivity of the Bonding Group. There is an extensive body of knowledge, well known to those skilled in the art of organic chemistry, that predicts the relative reactivity of possible Bonding Groups. This knowledge can be used to systematically alter and weaken a Bonding Group during the course of improvement of a Bifunctional Blocker. However, if the Specificity Group is connected to the Bonding Group in such a way that the Specificity Group's composition influences the Bonding Group's reactivity, then it will be difficult to systematically weaken the reactivity of the Bonding Group. It is therefore preferable to connect these Groups so that they do not influence each other.

The invention also features a compound having a molecular weight of less than 1500 daltons that covalently bonds to an amino acid side chain of a target protein that is not an enzyme; a covalently bound portion of the compound sterically blocks the binding of the target protein to a second protein. The compound bonds to the target protein with a forward reaction rate that is at least 100 times faster than the forward reaction rate at which the compound bonds to the side chain of the corresponding free amino acid under physiological conditions. A preferred compound bonds to the target protein with a forward reaction rate that is at least 1000 times faster than the forward reaction rate at which the compound bonds to the side chain of the free amino acid under physiological conditions, and more preferably bonds with a rate 10,000 times, or 100,000 times faster.

In this case, a preferred compound also has a covalent bonding rate constant with the side chain of the free amino acid of less than 10^{-5} /M/sec at

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room temperature under physiological conditions, and more preferably has a rate constant of less than 10^{-6} /M/sec, or 10^{-7} /M/sec at room temperature under physiological conditions. A preferred compound is one that is prepared synthetically.

5 In addition, a preferred compound includes a Specificity Group whose removal results in the bonding reaction rate with the side chain of the free amino acid being substantially unchanged, and the bonding reaction rate with the target protein being reduced to a rate that is substantially similar to the bonding reaction rate with the side chain of the free amino acid; the compound
10 also includes a Bonding Group that forms the covalent bond with the target protein. In this compound, modification of the Specificity Group does not substantially alter the bonding reactivity of the Bonding Group. Preferred target proteins include kinases, viral coat proteins, STAT proteins, oncogenes, transcription factors, and extracellular protein ligands, protein domains, and
15 their receptors. More specifically, preferred target proteins include MCP-1, Fos, and IL-1 beta.

The invention also features a substantially pure compound having a molecular weight of less than 1500 daltons that includes a Specificity Group that non-covalently interacts with the surface of a target protein and a Bonding
20 Group that covalently bonds to the target protein. A covalently bound portion of the compound competitively and sterically inhibits the binding of a second protein to the target protein.

The invention further features a combinatorial chemistry library containing at least 25 compounds. Each compound contains the same Bonding
25 Group, which covalently bonds to the side chains of amino acids. The covalent bonding rate constant is between 10^{-3} /M/sec and 10^{-7} /M/sec for the reaction of the Bonding Group with the side chain of the free amino acid that reacts most

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rapidly with the Bonding Group to give a stable product (i.e., a product that has a sufficiently long half-life in physiological conditions to inhibit the target protein usefully). In preferred libraries, each compound also includes a Specificity Group.

5 The invention further features a pharmaceutical composition including a compound having a molecular weight of less than 1500 daltons; the compound non-covalently interacts with, and covalently bonds to, a target protein at an amino acid side chain that is not part of an enzyme active site. A covalently bound portion of the compound sterically blocks the binding of the
10 target protein to a second protein. The compound bonds to the target protein with a forward reaction rate that is at least 100 times faster than the forward reaction rate at which the compound bonds to the side chain of the corresponding free amino acid under physiological conditions. The pharmaceutical composition also includes a pharmaceutically acceptable
15 carrier. The pharmaceutical compositions of the invention are free of contaminating substances, such as trace organic solvents and synthetic precursors, that would make the composition unacceptable for clinical use.

 In a preferred composition, the compound bonds to the target protein with a forward reaction rate that is at least 1000 times faster than the forward
20 reaction rate at which the compound bonds to the side chain of the free amino acid under physiological conditions, and more preferably bonds with a rate 10,000 times, or 100,000 times faster. In another preferred composition, the compound has a covalent bonding rate constant with the side chain of the free amino acid of less than 10^{-5} /M/sec at room temperature under physiological
25 conditions, and more preferably has a rate constant of less than 10^{-6} /M/sec, or 10^{-7} /M/sec at room temperature under physiological conditions.

 Another preferred composition includes a compound that includes a

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Specificity Group whose removal results in the bonding reaction rate with the side chain of the free amino acid being substantially unchanged, and the bonding reaction rate with the target protein being reduced to a rate that is substantially similar to the bonding reaction rate with the side chain of the free amino acid. In this composition, the compound includes a Bonding Group that forms the covalent bond with the target protein. Modification of the Specificity Group does not substantially alter the bonding reactivity of the Bonding Group. Preferred target proteins include kinases, viral coat proteins, STAT proteins, oncogenes, transcription factors, and extracellular protein ligands and their receptors. More specifically, preferred target proteins include MCP-1, Fos, and IL-1 beta. In a preferred composition, the Bonding Group forms a covalent bond with the amino acid side chain of the target protein as a result of the Bonding Group's intrinsic reactivity.

The invention also features a composition containing a compound having a molecular weight of less than 1500 daltons that non-covalently interacts with, and covalently bonds to, a target protein that is not an enzyme; a covalently bound portion of the compound sterically blocks the binding of the target protein to a second protein. The compound bonds to the target protein with a forward reaction rate that is at least 100 times faster than the forward reaction rate at which the compound bonds to the side chain of the corresponding free amino acid under physiological conditions. A preferred composition includes a compound that bonds to the target protein with a forward reaction rate that is at least 1000 times faster than the forward reaction rate at which the compound bonds to the side chain of the free amino acid under physiological conditions, and more preferably bonds with a rate 10,000 times, or 100,000 times faster.

In another preferred composition, the compound has a covalent

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bonding rate constant with the side chain of the free amino acid of less than 10^{-5} /M/sec at room temperature under physiological conditions, and more preferably has a rate constant of less than 10^{-6} /M/sec, or 10^{-7} /M/sec at room temperature under physiological conditions.

5 In addition, a preferred composition contains a compound that includes a Specificity Group whose removal results in the bonding reaction rate with the side chain of the corresponding free amino acid being substantially unchanged, and the bonding reaction rate with the target protein being reduced to a rate that is substantially similar to the bonding reaction rate with the side
10 chain of the free amino acid; this compound also includes a Bonding Group that forms the covalent bond with the target protein. Modification of the Specificity Group does not substantially alter the bonding reactivity of the Bonding Group. Preferred target proteins include kinases, viral coat proteins, STAT proteins, oncogenes, transcription factors, and extracellular protein
15 ligands, protein domains, and their receptors. More specifically, preferred target proteins include MCP-1, Fos, and IL-1 beta.

 The invention further features a method of producing a compound that selectively binds to a target protein. The method includes (a) starting with a candidate compound having a molecular weight of less than 1500 daltons and
20 including a first group and a second group, where the second group covalently bonds to an amino acid side chain of the target protein and the first group does not covalently bond to any amino acid side chain, but may non-covalently interact with the target protein; (b) testing the candidate compound for inhibition of the interaction of the target protein and a second protein ; (c)
25 testing the candidate compound for binding to a non-target molecule, such as an amino acid or non-target protein; (d) testing the candidate compound for bonding reactivity of the second group with the amino acid side chain of the

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target protein; (e) altering the first group and selecting for increased selectivity for the target protein, where increased selectivity is indicated by an increase in inhibition of the interaction of the target protein and a second protein, relative to binding to the non-target molecule; (f) altering the second group and
5 selecting for decreased bonding reactivity of the second group with the amino acid side chain of the target protein and with the non-target molecule; and (g) optionally repeating steps (e) and (f), to obtain a compound that has selectivity for the target protein, relative to the non-target molecule, at least 100 times greater than the candidate compound of step (a). Preferably, the compound
10 selectively binds to the target protein at an amino acid side chain that is not part of an enzyme active site, and also binds to the target protein at a site of protein-protein interaction. In a preferred method, the candidate compound is present in or chosen from a combinatorial chemistry library. In a different preferred method, the compound is designed by principles of rational drug design or with
15 the aid of a computer molecular modeling system. In another preferred method, the first group is a Specificity Group that non-covalently binds to the protein under physiological conditions, the second group is a Bonding Group that forms a covalent bond with the amino acid side chain on the protein, and modification of the Specificity Group does not substantially alter the reactivity
20 of the Bonding Group. In still another preferred method, the candidate compound of step (a) is a compound having a molecular weight of less than 1500 daltons that covalently bonds to a target protein at an amino acid side chain that is not part of an enzyme active site; a covalently bound portion of the compound sterically blocks the binding of the target protein to a second
25 protein. The candidate compound bonds to the target protein with a forward reaction rate that is at least 100 times faster than the forward reaction rate at which the compound bonds to the side chain of the corresponding free amino

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acid under physiological conditions.

The invention also features a method of producing a compound that selectively binds to a target protein; the method includes (a) starting with a candidate compound having a molecular weight of less than 1500 daltons and including a first group and a second group, where the second group covalently bonds to an amino acid side chain of the target protein and the first group does not covalently bond to any amino acid side chain, but may non-covalently interact with the target protein; (b) testing the candidate compound for inhibition of the interaction of the target protein and a second protein; (c) testing the candidate compound for binding to a non-target molecule, such as an amino acid or non-target protein; (d) testing the candidate compound for bonding reactivity of the second group with the amino acid side chain of the target protein; (e) altering the first group and selecting for increased selectivity for the target protein, where increased selectivity is indicated by an increase in inhibition of the interaction of the target protein and a second protein, relative to binding to the non-target molecule; (f) altering the second group and selecting for decreased bonding reactivity of the second group with the amino acid side chain of the target protein and with the non-target molecule; and (g) optionally repeating steps (e) and (f) to obtain a compound that has a selectivity for the target protein, relative to the non-target molecule, at least 100 times greater than the candidate compound of step (a) and that has a covalent bonding rate with the side chain of the corresponding free amino acid of less than 10^{-5} /M/sec at room temperature under physiological conditions. Preferably, the compound selectively binds to the target protein at an amino acid side chain that is not part of an enzyme active site, and also binds to the target protein at a site of protein-protein interaction.

The invention also features a method of producing a compound that

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selectively binds to a target protein including (a) starting with a candidate compound having a molecular weight of less than 1500 daltons and including a first group and a second group, where the second group covalently bonds to an amino acid side chain of the target protein and the first group does not

5 covalently bond to any amino acid side chain, but may non-covalently interact with the target protein; (b) testing the candidate compound for inhibition of the interaction of the target protein and a second protein; (c) testing the candidate compound for binding to a non-target molecule, such as an amino acid or non-target protein; (d) altering the first group and selecting for increased

10 selectivity for the target protein, where increased selectivity is indicated by an increase in inhibition of the interaction of the target protein and a second protein, relative to binding to the non-target molecule; (e) replacing the second group with a group known to be less reactive, altering the first group, and selecting for substantially unchanged or increased inhibition; and (f) optionally

15 repeating steps (d) and (e), to obtain a compound that has a selectivity for the target protein, relative to the non-target molecule, at least 100 times greater than the candidate compound of step (a). Preferably, the compound selectively binds to the target protein at an amino acid side chain that is not part of an enzyme active site, and also binds to the target protein at a site of protein-

20 protein interaction.

Finally, the invention features a method of sterically blocking protein-protein binding. The method includes contacting a target protein with a compound having a molecular weight of less than 1500 daltons, where the compound non-covalently interacts with, and covalently bonds to, the target

25 protein at an amino acid side chain that is not part of an enzyme active site; a covalently bound portion of the compound sterically blocks the binding of the target protein to a second protein. In addition, the compound bonds to the

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target protein with a forward reaction rate that is at least 100 times faster than the forward reaction rate at which the compound bonds to the side chain of the corresponding free amino acid. The compound has a covalent bonding rate constant with the side chain of the free amino acid of less than 10^{-5} /M/sec under physiological conditions. Preferred methods include those used for therapeutic or diagnostic purposes. Additional methods include those used for pesticidal or herbicidal purposes.

In preferred methods, the compounds bonds to the target protein with a forward reaction rate 1000 times faster than the rate at which the compound bonds to the side chain of the corresponding free amino acid, or 10,000, or 100,000 times faster. In other preferred methods, the compound has a covalent bonding rate constant with the side chain of the free amino acid of less than 10^{-6} /M/sec or 10^{-7} /M/sec under physiological conditions. In addition, in preferred methods, the compound has a Specificity Group and a Bonding Group, and modification of the Specificity Group does not substantially alter the reactivity of the Bonding Group. Preferably, this Bonding Group forms a covalent bond with the side chain of an amino acid of the target protein as a result of the intrinsic reactivity of the Bonding Group.

Preferably, the target protein is not an enzyme and/or the compound is a synthetic compound. Protein-protein binding can be blocked in a mammal, such a human. In preferred instances, the protein-protein binding that is blocked with this method causes a disease.

By a "Specificity Group" or "S group" is meant a moiety that non-covalently interacts with a target protein, causing a Bonding Group to have a high local concentration near an amino acid side chain on the target protein. The formation of a covalent bond between the Bonding Group and the side

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chain of the amino acid is accelerated.

An important characteristic of the Specificity Group is that its removal results in the bonding reaction rate with the side chain of an amino acid of a target protein being substantially reduced, and the bonding rate with the corresponding free amino acid being substantially unchanged.

Alternatively, removal of the Specificity Group from the compound results in the bonding reaction rate with the side chain of the free amino acid being substantially unchanged, and the bonding reaction rate with the amino acid side chain of the target protein being reduced to a rate that is substantially similar to the bonding reaction rate with that of the free amino acid.

By a "Bonding Group" or "B group" is meant a moiety of a compound that forms a covalent bond with an amino acid of a target protein. The Bonding Group does not substantially affect the non-covalent binding affinity of the compound for the target protein.

By "corresponding free amino acid" is meant a free amino acid that is the same amino acid as the amino acid of a target protein that forms a covalent bond with a compound of the invention.

By "protein" is meant a polypeptide having at least 40 amino acids.

By "Bifunctional Blocker" is meant a compound that has an S group and a B group, that reacts selectively with a target protein, and in which a covalently bound portion of the compound sterically hinders the interaction of the target protein with another protein.

By "room temperature" is meant $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$.

By "physiological solution conditions" are meant the conditions, e.g., temperature, pH, and concentration, that are present in the body of the treated organism, such as a human being or other animal, or plant.

By "standard lead-optimization procedures" are meant processes for

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drug development that begin with the identification of a lead compound in one or more assays for drug activity. The identification is followed by the synthesis of chemical variants of the lead compound, the testing of the variants in the assays, and the selection of compounds with improved activity and selectivity.

5 The steps of synthesis, testing, and selection are often repeated for several cycles.

By "reactive" is meant capable of undergoing covalent bond formation or cleavage on a time scale, at concentrations, and under conditions that are relevant to pharmaceutical development, or under physiological
10 conditions.

By "reactive group" is meant a chemical moiety that is reactive as a result of its intrinsic chemical properties, and that reacts without the action of enzymes, photons, or molecules that are not present under physiological conditions.

15 By "intrinsic reactivity" is meant the ability to undergo covalent bond formation or cleavage without the action of enzymes, photons, or molecules that are not present under physiological conditions.

By "non-target molecule" is meant any molecule that is used for purposes of comparison with a target molecule in examining the reactivity of a
20 reactive compound. An example of a non-target molecule is a free amino acid to which a reactive compound bonds; another example is a protein, other than the target protein, that contains the same amino acid in a solvent-accessible conformation.

By "protein-protein interaction" is meant contact between proteins
25 that primarily involves polypeptide regions, rather than moieties such as sugars or lipids that are attached to the surface of a protein.

By "synthetic" is meant a compound or composition made by, e.g.,

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recombinant techniques or synthetic organic techniques, rather than a compound isolated from a natural source.

By "substantially pure" is meant that a compound, such as a Bifunctional Blocker of the invention, has been separated from substances which naturally accompany it, or which are generated during its preparation or extraction. Such substances include organic solvents, reaction precursors, and other possible contaminants. Substances such as water, buffers, chemicals introduced to increase the stability of the compound, or chemicals added for formulation purposes are not considered contaminants. Preferably the compound is at least 80%, more preferably at least 95%, and most preferably at least 99%, by weight, free from other compounds, such as proteins, lipids, and other naturally-occurring molecules with which it is naturally associated. The purity of the compounds of the invention can be measured by any appropriate method, for example, gas chromatography analysis or high performance liquid chromatography analysis.

By a "disease" is meant a condition of a living organism which impairs normal functioning of the organism, or an organ or tissue thereof.

The present invention provides a number of advantages. For example, the compounds of the invention bind to a non-enzymatic protein or to a surface of an enzyme that is not part of its active site; a covalently bound portion of the compound sterically blocks the binding of that protein to another protein. These compounds contain two distinct chemical moieties: a Specificity Group (S group) that mediates non-covalent interactions with a target protein, and a Bonding Group (B group) that covalently bonds with an amino acid on the target protein. Modification of the S group has a relatively small effect on the bonding activity of the B group, and modification of the B group has a relatively small effect on the non-covalent binding affinity of the S

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group. As a result of these unique features, the compounds of the invention covalently bond to target proteins tightly enough to result in therapeutic utility, while at the same time bonding to non-target molecules in the body at a rate low enough to avoid drug allergies and other side effects that may result from non-specific interactions with non-target molecules. The methods of the invention provide an improvement over existing drug optimization techniques, in that they allow the independent optimization of distinct portions of compounds of the invention.

Other features and advantages of the invention will be apparent from the following detailed description thereof, and from the claims.

Brief Description of the Drawings

Fig. 1A is a schematic representation of an enzyme bound to its substrate; Fig. 1B is a schematic representation of an enzyme bound to an enzyme inhibitor; Fig. 1C is a schematic representation of two interacting proteins; and Fig. 1D is a schematic representation of a Bifunctional Blocker that is covalently bonded to a protein and that sterically blocks the binding of two proteins.

Figs. 2A-2C are schematic representations of different Bifunctional Blockers bound to a hypothetical target protein.

Figs. 3A-3C are schematic representations of different ways in which to alter the reactivity of a compound to nucleophilic attack.

Fig. 4 is a schematic representation of a compound that includes an S group and a B group.

Detailed Description

The invention features compounds, compositions, and methods for

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blocking protein-protein interactions. The compounds of the invention sterically hinder the interaction of a target protein with another protein by bonding to a surface of the target protein and by blocking protein-protein interaction. For example, as shown in Fig. 1D, the compound 6 is covalently
5 bound to a protein 4 via a covalent bond 7. The compound 6 blocks the binding of a second protein 5 to the target protein 4. Preferred compounds bind to target proteins more tightly than the protein's normal binding partners bind. At the same time, the preferred compounds do not significantly bind to non-target molecules. As such, the compounds of the invention strike a balance
10 between selectivity and reactivity.

Compounds that perform the above functions, referred to herein as Bifunctional Blockers or BB's, are molecules that contain a Specificity Group (S group) that mediates non-covalent affinity for the target protein, and a distinct Bonding Group (B group) that covalently bonds to the target protein.
15 The S group may also determine the orientation of the B group relative to its target amino acid. The S group can be varied without significantly affecting the bonding reactivity of the B group, and the B group can be varied without significantly affecting the non-covalent binding affinity of the S group to the target protein.

20 Bifunctional Blockers are developed as follows. Lead compounds are initially identified by standard methods of medicinal chemistry, such as by rational drug design or by mass screening of chemical compound libraries in assays for drug activity. Alternatively, a lead compound may be constructed by the addition of a reactive group to a small-molecule drug that non-covalently
25 binds to a target protein and sterically hinders the target protein's interaction with a second protein.

The lead compounds are then improved through a method called

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Bidirectional Optimization. By this method, the step of lead optimization of the S group is alternated with stepwise decreases in the covalent bonding reactivity of the B group. Lead optimization of the S group includes synthesizing chemical variants of the lead compound, testing the variants in the assays, and selecting compounds with improved activity and selectivity. Lead optimization often involves several cycles of improvement; it is common for each cycle to result in 2-fold to 50-fold improvements in affinity or selectivity for a given target. The concepts and methods of lead optimization are familiar to those skilled in the art of pharmaceutical development, and are documented in the medicinal chemistry literature.

The covalent bonding reactivity of the B group is modified by changing factors that control rates of covalent bond formation, which are known to those skilled in the art of organic chemistry. For example, the electronic influence of a substituent on the reactivity of a small molecule is described by the Hammett equation. The Hammett equation can therefore be used to predict how the reactivity of a small molecule will vary when a substituent, which does not form covalent bonds to a second reactant, is changed. Similarly, the effects of substituting a leaving group or introducing a sterically hindered group can be predicted, as described in Lowry and Richardson, *Mechanism and Theory in Organic Chemistry*, ch. 2, 4 (3d ed. 1987).

The S group and the B group can be linked with a spacer group, such as an alkyl chain, such that modification of the S group has little effect on the reactivity of the B group. For example, the phenyl ring of the substituent -C₆H₄COOH can be seen as the S group, and the carboxyl group can be seen as the B group. Substituents on the S group affect the ionization constant of the B group in benzoic acid. The effect of the substituents on the S group, however,

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are significantly decreased in the substituent $-C_6H_4CH_2COOH$, which has a methylene group (the spacer group) between the S and the B group. The insertion of an even longer alkyl chain can further decrease the effect of substituents on the S group on the B group. This example illustrates one particular way in which the selectivity of the S group and the bonding reactivity of the B group may be relatively or completely independent of each other.

Fig. 2 illustrates a simple example of Bidirectional Optimization. Fig. 2A shows a non-optimized, highly reactive Bifunctional Blocker with an S group 12 that binds to a target protein 13 in such a way that the B group 14 is positioned to bond covalently with an amino acid side chain. Fig. 2B shows a Bifunctional Blocker with a B group 15 that is less reactive than the B group 14 shown in Fig. 2A; and Fig. 2C shows a Bifunctional Blocker that has an S group 16 that has a higher affinity for the target protein 13 than the S group 12 shown in Fig. 2A.

In its simplest form, Bidirectional Optimization proceeds as follows.

(1) A candidate compound is selected using procedures known in the art. For example, combinatorial chemistry can be used to generate large numbers of compounds that can then be screened for drug activity using the methods of, e.g., Hogan, *Nature Biotechnology* 15: 328-330 (1997); Janda et al., *Methods Enzymol.* 267:234-247 (1996); or Gordon, *Curr. Opin. Biotechnol.* 6(6): 624-631 (1995) can be used. Alternatively, variations on the computer-based method of Li et al., *Proc. Nat. Acad. Sci.* 94: 73-78 (1997) can be used to identify an initial candidate compound with a desired activity.

The candidate compound is tested for its inhibitory activity (e.g., an IC_{50} value) against the desired target protein in an assay, such as an *in vivo* or *in vitro* protein-protein binding assay. The compound is also tested for activity against non-target proteins or free amino acids in parallel assays. The assay of

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the non-target proteins can measure, for example, inhibition of protein-protein interaction or inhibition of cell growth.

The overall activity of the compound is determined by two parameters: the affinity of the S group for the target protein, and the reactivity of the B group with the side chain of an amino acid. These two parameters are altered in Bidirectional Optimization. However, because they cannot always be measured directly, these parameters are sometimes inferred from measurable quantities. The reactivity of the B group with the side chain of an amino acid can be measured directly; alternatively, it can be inferred by assaying the compound's effect on the interaction between non-target proteins containing this amino acid. The affinity of the S group for the target protein cannot be measured directly. Instead, what is measured is the ability of a given Bifunctional Blocker to inhibit the interaction between the target protein and a second protein. The overall activity of the Bifunctional Blocker is then inferred from this inhibiting activity. The affinity of the S group can in turn be inferred from the overall activity of the compound, and from the reactivity of the B group.

(2) Derivatives of the candidate compound selected in Step (1), in which the S group is varied but the B group remains unchanged, are then prepared. These derivatives are made by combinatorial chemistry techniques, by rational drug design methodologies, or by any other method of chemical design and synthesis familiar to those skilled in the art of pharmaceutical lead compound optimization. The derivatives are assayed against the target protein and non-target proteins as described in Step (1). Derivatives that exhibit increased activity against the target protein, and exhibit unchanged or decreased activity against the non-target proteins, are chosen for further development.

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(3) The compounds selected in Step (2) are further modified by decreasing the reactivity of the Bonding Group. These decreases are made in accordance with well-established rules of chemical bond formation, as described in Lowry and Richardson, *Mechanism and Theory in Organic Chemistry*, ch. 2, 4 (3d ed. 1987). For example, the steps of changing the leaving group from Br to Cl (shown in Fig. 3A) or making the compound more sterically hindered (shown in Fig. 3B) each make a compound less reactive toward nucleophilic attack. In addition, adding an electron-withdrawing group (shown in Fig. 3C) or an electron donating group can make the compound less reactive toward nucleophilic attack.

Based on the documented reactivity of chemically reactive groups, the reactivity of the B group can be decreased in increments of 3-fold to 20-fold, or more. The inhibitory activity of the compound in both the target assay and the non-target assay is decreased by about the same amount. Compounds with decreased reactivity are then selected for further optimization.

When the B group is changed, the quantitative effect on reactivity can often be predicted with some precision. For this reason, it is often practical to change and decrease the reactivity of the B group at the same time that the S group is varied.

(4) The selected compounds from Step (3) are then subjected to further rounds of Bidirectional Optimization. The above steps need not be performed in strict alternation. For example, a round of alteration of the S group may not yield sufficient improvement; in this case, the S group is modified again before the B group is altered.

Bidirectional Optimization is continued until a compound is obtained that has the desired selectivity for the target protein and non-reactivity for non-

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target molecules.

The B group of a Bifunctional Blocker forms a covalent bond with a side chain of an amino acid on the target protein to form a stable product. A stable product has a sufficiently long half-life in physiological conditions that
5 useful inhibition of the target protein results. Thus, the rate of reaction of the Bifunctional Blocker and the amino acid side chain of the target protein is not the important factor; the important issue is that the two react to give a stable product. For example, some amino acid side chains, such as cysteine, react quickly; these side chains, however, give relatively unstable products when
10 reacted with B Groups such as esters, aldehydes, and ketones.

Bifunctional Blockers are able to bind selectively to target proteins because the S group brings the B group into close proximity with the target protein surface. The B group is thus physically constrained to be near the amino acid with which it reacts. The effective concentration of a physically
15 constrained reactive group can be as high as 10^5 to 10^7 M in the neighborhood of its target group, leading to a significant enhancement of the reaction rate. This high effective concentration results from several factors, including physical proximity, loss of rotational entropy, and loss of translational entropy. The Bifunctional Blockers described in this application bring reactive groups
20 into close physical proximity, and result in a loss of translational entropy. A conservative estimate is that a typical effective concentration of a reactive B group, when part of a protein-bound Bifunctional Blocker, is about 10^3 M.

In addition to being selective for the target protein, the Bifunctional Blocker must be sufficiently non-reactive with non-target molecules that
25 significant adverse side effects, such as drug allergy, do not result. The desired level of reactivity can be determined using, as a model, a compound known to occasionally cause drug allergy. One particular example of such a compound is

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penicillin, which is well-known to cause allergic symptoms in some patients. Penicillin modifies blood proteins non-specifically by covalently bonding to lysine, and, as a result, certain individuals in a population suffer an allergic reaction to this drug. The vast majority of the population, however, does not
5 have such an allergic response. The forward reaction rate of penicillin with lysine side chains in proteins, which is about 1×10^{-5} /M/sec in physiological conditions, can therefore be considered an approximate upper limit for a safe level of reactivity with a non-target protein.

In sum, using the technique of Bidirectional Optimization,
10 Bifunctional Blockers that bind tightly to target proteins, yet are sufficiently unreactive with non-target molecules to avoid side effects such as drug allergy, may be synthesized. These compounds can be formulated into pharmaceutical compositions using techniques known to those skilled in the art.

There now follow particular examples of Bifunctional Blockers and
15 Bidirectional Optimization according to the invention. These examples are provided for the purpose of illustrating the invention, and should not be construed as limiting.

Example 1: Inhibition of the Fos/Jun Interaction

Fos and Jun are inducible DNA-binding proteins that turn on
20 transcription as a final step in many different signal transduction pathways. For example, the transcription of Fos is activated when Nerve Growth Factor is added to PC12 cells. Nerve Growth Factor is a stimulator of pain sensation in adult mammals; blocking Fos function is therefore a useful approach to the treatment of chronic pain. Fos has a cysteine thiol group, which reacts with
25 alkyl halides on or near its surface of interaction with Jun; covalent attachment of a typical drug-sized molecule to this group would therefore disrupt Fos/Jun

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interactions.

A Bifunctional Blocker for this purpose is developed as follows. A family of candidate compounds is synthesized by combinatorial chemistry using, for example, the method of Hogan, *Nature Biotechnology* 15:328-330 (1997); Janda et al., *Methods Enzymol.* 267:234-247 (1996); or Gordon, *Curr. Opin. Biotechnol.* 6(6): 624-631 (1995) to generate S groups. The B group of these compounds is preferably an alpha-bromo carboxylic acid, which is expected to undergo nucleophilic attack by the cysteine thiol group with a forward rate constant of about 8×10^{-5} /M/sec. A B group is attached to each of the S groups of the compounds by a straight alkyl chain of any appropriate length. An example of such a compound is [S group]-CH₂CH₂CH₂CHBrCOOH.

The activity of each candidate compound is assayed, for example, as follows. Fos is bound to the bottom of a microtiter plate by standard procedures. Each candidate compound is added to a final concentration of 1 μ M and incubated with the Fos protein for 45 minutes. The plate wells are washed to remove unbound compounds. Fluoresceinated Jun protein and a DNA oligonucleotide comprising a Fos/Jun binding site are then added. The mixture is incubated for an additional 30 minutes; the wells are washed, and the binding of fluoresceinated Jun is measured. Compounds that cause at least 90% inhibition are examined for further study. Alternatively, a variation of the gel-shift assay used by Abate et al., *Science* 249: 1157-1161 (1990), or a variation of the assay for protein-protein interaction of Devos et al., *European Journal of Biochemistry* 225: 635-640 (1994), may be used to assay Fos/Jun interactions.

For a provided candidate compound, the affinity of the S group for Fos can be expected to be $K_{\text{affinity}} = 10^4$ /M. When the compound is

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non-covalently bound to a protein, the effective concentration of its B group in the range of the target cysteine is likely to be at least 10^3 M. (Although in practice, the affinity of the S group and the effective concentration of the B group are often not determinable as separate quantities.) The fractional

5 occupancy is the extent to which the compound, present in excess and under non-saturating conditions (i.e., at a concentration of 10^{-6} M), non-covalently binds to the target protein, and is given by the following equation:

$$\text{fractional occupancy} = [\text{concentration of Blocker}] \times K_{\text{affinity}} = 10^{-6}\text{M} \times 10^4/\text{M}$$

Under these circumstances, the forward rate (k) of covalent bonding is

10 (fractional occupancy) x (effective concentration with full occupancy) x (forward rate constant for compound with free cysteine) x [protein concentration] = $0.01 \times 1000 \text{ M} \times 0.0001/\text{M}/\text{sec} \times [\text{protein conc.}] = 10^{-3}/\text{sec} \times [\text{protein concentration}]$.

The proportion of unreacted protein that remains after time t is given by (e^{-kt}) , where k is the forward rate of covalent bonding and t is time in seconds. It

15 follows that the amount of covalently bonded protein is $(1 - e^{-kt})$. Thus, the rate calculated using the above equation indicates that, in 45 minutes (2700 seconds), the provided candidate compound covalently bonds to $(1 - e^{-2.7})$, or 95%, of the target protein, and causes a 95% inhibition in the protein

20 interaction assay described above.

The $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CHBrCOOH}$ B group is replaced with a $-\text{CH}_2\text{CH}_2\text{CH}_2\text{C}(\text{CH}_3)\text{BrCOOH}$ B group, which is expected to be about 15-fold less reactive. At the same time, derivatives of the S group are attached to the

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new B group. The new compounds are assayed as described above. Due to the decreased reactivity of the new B group, the S group must be able to bind at least 7-fold more tightly than the previous S group to achieve 90% inhibition in the assay. If an improvement of less than 7-fold in the binding constant of the S group is achieved, less than 90% inhibition will be achieved. In this case, further modifications of the S group are made before the B group is altered again.

In the second round of Bidirectional Optimization, the -CH₂CH₂CH₂C(CH₃)BrCOOH B group is replaced with a -CH₂CH₂CH₂CHClCOOH B group, which is expected to be about 15-fold less reactive than the -CH₂CH₂CH₂C(CH₃)BrCOOH B group. At the same time, derivatives of the S group are synthesized and attached to the -CH₂CH₂CH₂CHClCOOH B group. The compounds are assayed as described above.

A compound containing an S group that, in combination with the -CH₂CH₂CH₂CHClCOOH B group, causes the same inhibition of Fos-Jun interaction, represents about a 200-fold improvement in the Bifunctional Blocker. This improved compound has about the same reactivity for the target protein as the starting compound, but the selectivity of the compound is improved by about 200-fold.

A Bifunctional Blocker with a -CH₂CH₂CH₂CHClCOOH B group has a non-specific covalent bonding rate with proteins of about 4x10⁻⁷/M/sec, which is significantly lower than the covalent bonding rate for penicillin; this compound is therefore potentially useful as a drug.

Example 2: Inhibition of the IL-1/IL-1R Interaction

The IL-1 beta protein interacts with the IL-1 receptor (IL-1R) to

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stimulate inflammation. Disruption of this process is therefore a useful approach for treating a number of inflammatory diseases. There is a solvent-exposed methionine residue (Met36) in IL-1 beta near its interaction surface with IL-1R, and attachment of a drug-sized molecule to this residue is
5 expected to disrupt the interaction with IL-1R.

In this particular example, a provided compound with a B group consisting of 2-methoxy-5-nitrobenzyl bromide, shown in Fig. 4, is preferably used as the starting point. 2-Methoxy-5-nitrobenzyl bromide covalently bonds to tryptophan, methionine, and cysteine residues in proteins. The rate of
10 reaction with methionine and tryptophan is similar, and is about $1.5-3 \times 10^{-3}/\text{M}/\text{sec}$. The reaction occurs by an $\text{S}_{\text{N}}1$ mechanism; the reaction rate is therefore relatively insensitive to the reactivity of the nucleophilic amino acid, and instead depends on the reactivity of the electrophile. The reactivity of 2-methoxy-5-nitrobenzyl bromide is strongly influenced by the electronic
15 effects of substituents on the benzene ring. The addition of electron-withdrawing groups, such as halides, decreases the rate of $\text{S}_{\text{N}}1$ reactions.

To illustrate the power of the Bidirectional Optimization method, a candidate compound including an S group having a non-covalent affinity of
20 only $K_{\text{affinity}} = 10^3/\text{M}$ for IL-1 beta is used in this example. The reactivity of the candidate compound including 2-methoxy-5-nitrobenzyl bromide will be about the same as the reactivity of 2-methoxy-5-nitrobenzyl bromide itself, because the electronic effect of the S group is small. Therefore, the candidate compound has a non-specific reaction rate with exposed methionine side chains
25 of about $1 \times 10^{-3}/\text{M}/\text{sec}$.

The interaction of IL-1 beta with the soluble form of IL-1R is

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assayed in a protein-based assay similar to those described above, using the same protein concentrations and incubation times as described above.

When the compound is non-covalently bound to IL-1 beta, there is likely to be an effective concentration of at least 10^3 M of the

- 5 2-methoxy-5-nitrobenzyl bromide group in the neighborhood of Met36. In this example, the fractional occupancy is $10^{-6}\text{M} \times 10^3/\text{M} = 0.001$. According to the equation of Example 1, the forward rate of production of covalently bonded protein is $10^{-3} \times [\text{protein concentration}]$.

- Bidirectional Optimization is used to reduce the non-specific
10 reactivity of the compound to equal to or less than that of penicillin as follows. The 2-methoxy-5-nitrobenzyl bromide group is replaced by a similar group with a fluorine in the 6 (ortho) position; this replacement reduces reactivity by about 20-fold. At the same time, variants of the S group are attached to this new, less reactive B group, and the new compounds are screened for activity.
15 The compounds that are most effective in blocking the IL-1 beta/IL-1R interaction are chosen for further optimization. These compounds are also checked for reduced reactivity with free methionine and with non-target molecules.

A second step of Bidirectional Optimization proceeds as follows.

- 20 The 2-methoxy-5-nitro-6-fluorobenzyl bromide B group is replaced with 2-methoxy-5-nitro-6-bromobenzyl bromide; this replacement depresses the reaction rate even further. As described above, the S group is further modified. The most active compounds are then tested for reduced reactivity against free methionine and against non-target proteins.

- 25 An alternative way to use Bidirectional Optimization to reduce the non-specific reactivity of the compound to equal to or less than that of penicillin is to introduce a bromine at the 4 position of the benzene ring (an

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~5-fold effect), or to replace the bromine leaving group with a chlorine (an ~40-fold effect). These effects are summarized in Tables 1 and 2. The non-specific reactivity of the compound will then be less than 10^{-5} /M/sec, which is comparable to the reactivity of penicillin for amino acid side chains in proteins.

Table 1. Effects of halogen substitutions of the phenyl ring on S_N1 reaction rates.

	Relative reaction rate
R = H	1.0
R = F	0.05
R = Br	0.006
R' = H	1.0
R' = Br	0.2

Table 2. Effects of the leaving group on S_N1 reaction rates.

Leaving group	Relative reaction rate
Br	1
Cl	0.025

Example 3: Inhibition of the MCP-1/CCR2 Interaction

Monocyte/Macrophage Chemotactic Protein-1 (MCP-1), a chemokine released by inflamed cells, attracts monocytes and macrophages to sites of tissue damage. Its receptor, CC-Chemokine Receptor 2 (CCR2), is present on the surface of monocytes and macrophages. Both of these proteins are thought to play a role in the development of atherosclerosis.

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Atherosclerosis develops when macrophages migrate into the subendothelial space in a blood vessel, take up cholesterol, and turn into "foam cells," which then die and leave behind cholesterol crystals. The inhibition of the interaction of MCP-1 and CCR2 is therefore potentially useful for controlling

5 atherosclerosis and other inflammatory diseases.

The three-dimensional structure of MCP-1 has been determined. It is thought that amino acids 22 to 34 play a key role in the interaction between MCP-1 and CCR2. Possible targets for covalent modification include Lys44, which is adjacent to the stretch of amino acids 22 to 34, Ser27, and Ser34.

10 A Bifunctional Blocker that binds to MCP-1, thereby disrupting its interaction with CCR2, is developed as follows. A compound that is known to bind non-covalently to the MCP-1 pocket, such as a peptide segment from CCR2, is used as a Specificity Group. Alternatively, an S group is chosen using conventional screens that identify compounds that inhibit the

15 MCP-1/CCR2 interaction. In one particular example, the provided compound has an S Group with a predicted non-covalent affinity for MCP-1 of $K_{\text{affinity}} = 10^3/\text{M}$ ($K_{\text{dissociation}} = 1 \text{ mM}$).

A Bonding Group, such as the aspirin derivative 3,5-dibromoacetylsalicylic acid, is linked to the Specificity Group to construct

20 a first-generation Bifunctional Blocker that covalently bonds to MCP-1. The B Group is attached to the S Group by an alkyl chain of any length. For example, an alkyl chain having between 2 and 12 carbon atoms may be used. An example of such a compound is 4-[S group]-3,5-dibromoacetylsalicylic acid. Such a compound has a reactivity with free lysine side chains of about $1 \times 10^{-3}/\text{M}/\text{sec}$, at 37°C , pH 7.5.

25

The candidate Bifunctional Blocker is incubated, at a concentration of $1 \mu\text{M}$, for 45 minutes at 37°C at pH 7.5 with purified ^{125}I -labeled MCP-1.

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The inhibition of MCP-1 activity is assayed, for example, according to the procedure of Ernst et al. (*J. Immunol.* 152:3541-3549 (1994)). In this procedure, ^{125}I -labeled MCP-1 is added to elutriated monocytes, which express the CCR2 receptor in their plasma membranes. Alternatively, an extract of the monocyte cell membranes is made and adhered to microtiter wells, according to standard procedures.

When the compound is non-covalently bound to MCP-1, the effective concentration of its B group in the range of a target lysine is calculated to be at least 10^3 M . The fractional occupancy of the Bifunctional Blocker on MCP-1 is given by the following equation:

Fractional occupancy = concentration of Blocker $\times K_{\text{affinity}} = 10^{-6} \text{ M} \times 10^3/\text{M}$. Under these circumstances, the forward rate (k) of covalent bonding is (fractional occupancy) \times (effective concentration with full occupancy) \times (forward rate constant for compound with free lysine) \times [protein concentration] = $0.001 \times 1000 \text{ M} \times 0.001/\text{M}/\text{sec} \times [\text{protein concentration}] = 10^{-3}/\text{sec} \times [\text{protein concentration}]$.

The proportion of unreacted protein that remains after time t is given by (e^{-kt}) , where k is the forward rate of covalent bonding and t is the time in seconds. Thus, the rate calculated using the above equation indicates that, in 45 minutes (2700 seconds), such a compound covalently bonds to $(1-e^{-kt})$, or 95%, of the target protein, and causes a 95% inhibition in the protein interaction assay described above.

The 3,5-dibromoacetylsalicylic acid B group is replaced with an acetylsalicylic acid (aspirin) B group, which is expected to be approximately 8-fold less reactive. At the same time, derivatives of the S group, generated by standard medicinal chemistry techniques, are attached to the new B group. The new compounds are assayed as described above. Because of the decreased

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reactivity of the new B group, the S group must be able to bind at least about 4-fold more tightly to achieve 90% inhibition in the assay. If an improvement of less than 4-fold in the binding constant of the S group is achieved, less than 90% inhibition will be achieved. In this case, further modifications of the S
5 group are made before the B group is altered again.

A compound containing a S group that, in combination with the acetylsalicylic acid B group, is an effective inhibitor of MCP-1/CCR-2 interaction. At the same time, this Bifunctional Blocker has approximately the non-specific reactivity of aspirin, a compound that has been shown to be quite
10 safe.

The use of acylating agents such as aspirin, penicillin, and their derivatives helps avoid the modification of important cysteine-containing non-target proteins. Cysteine has the most reactive of all the amino acid side chains. Because the side chain of cysteine has a much more reactive
15 nucleophile than, for example, the side chain of lysine or serine, cysteines on important proteins can become substantially modified. When these cysteines are altered, the corresponding proteins become inhibited, resulting in undesired side effects. When aspirin is used as an acylating agent, however, the acylation of cysteine results in an unstable thiol ester that spontaneously hydrolyzes to
20 regenerate cysteine. The cysteine-containing proteins therefore remain substantially unaltered.

Example 4: Preparation of Combinatorial Chemistry Libraries

Libraries of compounds may be synthesized using combinatorial chemistry techniques, in which a particular B group is incorporated into each
25 member of the library. For convenience, a Bonding Group with a reactivity about the same as that of penicillin is preferably employed.

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The strategies and techniques for generating such combinatorial libraries are familiar to those skilled in the art of medicinal chemistry. To incorporate a reactive Bonding Group into the library, it is necessary to add the reactive Bonding Group in a protected form, to add the Bonding Group in the
5 final synthetic step, or to take some other precaution to ensure that the Bonding Group does not undergo a reaction during construction of the library. The specific strategies and techniques to accomplish such a synthesis are familiar to those skilled in the art of medicinal chemistry.

All publications and patents mentioned in this specification are
10 herein incorporated by reference to the same extent as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

Other Embodiments

From the foregoing description, it will be apparent that variations
15 and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

What is claimed is:

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Claims

1. A substantially pure compound having a molecular weight of less than 1500 daltons, said compound non-covalently interacting with a target protein and covalently bonding to said target protein at an amino acid side chain that is not part of an enzyme active site, a covalently bound portion of said compound sterically blocking the binding of said target protein to a second protein, wherein said compound bonds to said target protein with a forward reaction rate that is at least 100 times faster than the forward reaction rate at which said compound bonds to the side chain of the corresponding free amino acid under physiological conditions.
2. The compound of claim 1, wherein said compound bonds to said target protein with a forward reaction rate that is at least 1000 times faster than the forward reaction rate at which said compound bonds to the side chain of said free amino acid under physiological conditions.
3. The compound of claim 1, wherein said compound bonds to said target protein with a forward reaction rate that is at least 10,000 times faster than the forward reaction rate at which said compound bonds to the side chain of said free amino acid under physiological conditions.
4. The compound of claim 1, wherein said compound bonds to said target protein with a forward reaction rate that is at least 100,000 times faster than the forward reaction rate at which said compound bonds to the side chain of said free amino acid under physiological conditions.
5. The compound of claim 1, wherein said compound has a covalent

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bonding rate constant with the side chain of said free amino acid of less than 10^{-5} /M/sec at room temperature under physiological conditions.

6. The compound of claim 1, wherein said compound has a covalent bonding rate constant with the side chain of said free amino acid of less than 10^{-6} /M/sec at room temperature under physiological conditions.

7. The compound of claim 1, wherein said compound has a covalent bonding rate constant with the side chain of said free amino acid of less than 10^{-7} /M/sec at room temperature under physiological conditions.

8. The compound of claim 1, wherein said compound comprises (a) a Specificity Group whose removal results in the bonding reaction rate with said side chain of said free amino acid being substantially unchanged, and the bonding reaction rate with said target protein being reduced to a rate that is substantially similar to the bonding reaction rate with said side chain of said free amino acid; and (b) a Bonding Group that forms the covalent bond with said target protein, wherein modification of said Specificity Group does not substantially alter the bonding reactivity of said Bonding Group.

9. The compound of claim 8, wherein said Bonding Group forms said covalent bond with said amino acid on said target protein as a result of the intrinsic reactivity of said Bonding Group.

10. The compound of claim 1, wherein said target protein is not an enzyme.

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11. The compound of claim 10, wherein said compound bonds to said target protein with a forward reaction rate that is at least 1000 times faster than the forward reaction rate at which said compound bonds to said side chain of said free amino acid under physiological conditions.

5 12. The compound of claim 10, wherein said compound has a covalent bonding rate constant with said side chain of said free amino acid of less than 10^{-5} /M/sec at room temperature under physiological conditions.

13. The compound of claim 10, wherein said compound has a covalent bonding rate constant with said side chain of said free amino acid of
10 less than 10^{-6} /M/sec at room temperature under physiological conditions.

14. The compound of claim 10, wherein said compound has a covalent bonding rate constant with said side chain of said free amino acid of less than 10^{-7} /M/sec at room temperature under physiological conditions.

15 15. The compound of claim 1, wherein said compound is a synthetic compound.

16. A substantially pure compound having a molecular weight of less than 1500 daltons, said compound comprising a Specificity Group that non-covalently interacts with the surface of a target protein and a Bonding Group that covalently bonds to said target protein, a covalently bound portion
20 of said compound competitively and sterically inhibiting the binding of a second protein to said target protein.

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17. A combinatorial chemistry library containing at least 25 compounds, each compound comprising the same Bonding Group, said Bonding Group covalently bonding to the side chains of amino acids, wherein the covalent bonding rate constant is between 10^{-3} /M/sec and 10^{-7} /M/sec for the reaction of said Bonding Group with the side chain of the free amino acid that reacts most rapidly with said Bonding Group to give a stable product.

18. The library of claim 17, wherein each compound comprises a Specificity Group.

19. A pharmaceutical composition comprising (a) a substantially pure compound having a molecular weight of less than 1500 daltons, said compound non-covalently interacting with a target protein and covalently bonding to said target protein at an amino acid side chain that is not part of an enzyme active site, a covalently bound portion of said compound sterically blocking the binding of said target protein to a second protein, wherein said compound bonds to said target protein with a forward reaction rate that is at least 100 times faster than the forward reaction rate at which said compound bonds to the side chain of the corresponding free amino acid under physiological conditions, and (b) a pharmaceutically acceptable carrier.

20. The composition of claim 19, wherein said compound bonds to said target protein with a forward reaction rate that is at least 1000 times faster than the forward reaction rate at which said compound bonds to said side chain of said free amino acid under physiological conditions.

21. The composition of claim 19, wherein said compound bonds to

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said target protein with a forward reaction rate that is at least 10,000 times faster than the forward reaction rate at which said compound bonds to said side chain of said free amino acid under physiological conditions.

22. The composition of claim 19, wherein said compound bonds to
5 said target protein with a forward reaction rate that is at least 100,000 times faster than the forward reaction rate at which said compound bonds to said side chain of said free amino acid under physiological conditions.

23. The composition of claim 19, wherein said compound has a
covalent bonding rate constant with said side chain of said free amino acid of
10 less than 10^{-5} /M/sec at room temperature under physiological conditions.

24. The composition of claim 19, wherein said compound has a
covalent bonding rate constant with said side chain of said free amino acid of
less than 10^{-6} /M/sec at room temperature under physiological conditions.

25. The composition of claim 19, wherein said compound has a
15 covalent bonding rate constant with said side chain of said free amino acid of
less than 10^{-7} /M/sec at room temperature under physiological conditions.

26. The composition of claim 19, wherein said compound comprises
(a) a Specificity Group whose removal results in the bonding reaction rate of
the compound with said side chain of said free amino acid being substantially
20 unchanged, and the bonding reaction rate of the compound with said target
protein being reduced to a rate that is substantially similar to the bonding rate
with said side chain of said free amino acid; and (b) a Bonding Group that

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forms a covalent bond with said amino acid on said target protein, wherein modification of said Specificity Group does not substantially alter the bonding reactivity of said Bonding Group.

27. The composition of claim 26, wherein said Bonding Group
5 forms said covalent bond with said amino acid on said target protein as a result of the intrinsic reactivity of said Bonding Group.

28. The composition of claim 19, wherein said target protein is not an enzyme.

29. The composition of claim 28, wherein said compound bonds to
10 said target protein with a forward reaction rate that is at least 1000 times faster than the forward reaction rate at which said compound bonds to said side chain of said free amino acid under physiological conditions.

30. The composition of claim 28, wherein said compound has a covalent bonding rate constant with said side chain of said free amino acid of
15 less than 10^{-5} /M/sec at room temperature under physiological conditions.

31. The composition of claim 28, wherein said compound has a covalent bonding rate constant with said side chain of said free amino acid of less than 10^{-6} /M/sec at room temperature under physiological conditions.

32. The composition of claim 28, wherein said compound has a
20 covalent bonding rate constant with said side chain of said free amino acid of less than 10^{-7} /M/sec at room temperature under physiological conditions.

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33. A method of producing a compound that selectively binds to a target protein, said method comprising

- (a) providing a candidate compound having a molecular weight of less than 1500 daltons and comprising a first group and a second group,
5 wherein said second group covalently bonds to an amino acid side chain of said target protein and said first group does not covalently bond to any amino acid side chain;
- (b) testing said candidate compound for inhibition of the interaction of said target protein and a second protein;
- 10 (c) testing said candidate compound for binding to a non-target molecule;
- (d) testing said candidate compound for bonding reactivity of said second group with said amino acid side chain of said target protein;
- (e) altering said first group and selecting for increased selectivity for
15 said target protein, wherein increased selectivity is indicated by an increase in the inhibition of the interaction of the target protein and said second protein, relative to said binding to said non-target molecule;
- (f) altering said second group and selecting for decreased bonding reactivity of said second group with said amino acid side chain of said target
20 protein and with the non-target molecule; and
- (g) optionally repeating steps (e) and (f), whereby a compound is obtained that has a selectivity for the target protein, relative to the non-target molecule, that is at least 100 times greater than said candidate compound of step (a).

- 25 34. The method of claim 33, wherein said compound selectively binds to said target protein at an amino acid side chain that is not part of an

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enzyme active site, and wherein said compound binds to said target protein at a site of protein-protein interaction.

35. The method of claim 33, wherein said candidate compound is present in or chosen from a combinatorial chemistry library.

5 36. The method of claim 33, wherein said first group is a Specificity Group that non-covalently binds to said protein under physiological conditions, and said second group is a Bonding Group that forms a covalent bond with said amino acid side chain on said protein, wherein modification of said Specificity Group does not substantially alter the reactivity of said Bonding Group.

10 37. The method of claim 33, wherein said candidate compound of step (a) is the compound of claim 1.

38. A method of producing a compound that selectively binds to a target protein, said method comprising

15 (a) providing a candidate compound having a molecular weight of less than 1500 daltons and comprising a first group and a second group, wherein said second group covalently bonds to an amino acid side chain of said target protein and said first group does not covalently bond to any amino acid side chain;

20 (b) testing said candidate compound for inhibition of the interaction of said target protein and a second protein;

 (c) testing said candidate compound for binding to a non-target molecule;

 (d) testing said candidate compound for bonding reactivity of said

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second group with said amino acid side chain of said target protein;

(e) altering said first group and selecting for increased selectivity for said target protein, wherein increased selectivity is indicated by an increase in the inhibition of the interaction of the target protein and said second protein,
5 relative to said binding to said non-target molecule;

(f) altering said second group and selecting for decreased bonding reactivity of said second group with said amino acid side chain of said target protein and with the non-target molecule; and

(g) optionally repeating steps (e) and (f), whereby a compound is
10 obtained that has a selectivity for the target protein, relative to the non-target molecule, that is at least 100 times greater than said candidate compound of step (a), wherein said compound has a covalent bonding rate with the side chain of the corresponding free amino acid of less than 10^{-5} /M/sec at room temperature under physiological conditions.

15 39. The method of claim 38, wherein said compound selectively binds to said target protein at an amino acid side chain that is not part of an enzyme active site, and wherein said compound binds to said target protein at a site of protein-protein interaction.

20 40. A method of producing a compound that selectively binds to a target protein, said method comprising

(a) providing a candidate compound having a molecular weight of less than 1500 daltons and comprising a first group and a second group, wherein said second group covalently bonds to an amino acid side chain of said target protein and said first group does not covalently bond to any amino acid
25 side chain;

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(b) testing said candidate compound for inhibition of the interaction of said target protein and a second protein;

(c) testing said candidate compound for binding to a non-target molecule;

5 (d) altering said first group and selecting for increased selectivity for said target protein, wherein increased selectivity is indicated by an increase in the inhibition of the interaction of the target protein and said second protein, relative to said binding to said non-target molecule;

 (e) replacing said second group with a group known to be less
10 reactive, altering said first group, and selecting for substantially unchanged or increased inhibition; and

 (f) optionally repeating steps (d) and (e), whereby a compound is obtained that has a selectivity for the target protein, relative to the non-target molecule, that is at least 100 times greater than said candidate compound of
15 step (a).

41. The method of claim 40, wherein said compound selectively binds to said target protein at an amino acid side chain that is not part of an enzyme active site, and wherein said compound binds to said target protein at a site of protein-protein interaction.

20 42. A method of sterically blocking protein-protein binding, the method comprising contacting a target protein with a substantially pure compound having a molecular weight of less than 1500 daltons, said compound non-covalently interacting with a target protein and covalently bonding to said target protein at an amino acid side chain that is not part of an enzyme active
25 site, a covalently bound portion of said compound sterically blocking the

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binding of said target protein to a second protein, wherein said compound bonds to said target protein with a forward reaction rate that is at least 100 times faster than the forward reaction rate at which said compound bonds to the side chain of the corresponding free amino acid, said compound having a
5 covalent bonding rate constant with said side chain of said free amino acid of less than 10^{-5} /M/sec under physiological conditions.

43. The method of claim 42, wherein said compound bonds to said target protein with a forward reaction rate that is at least 1000 times faster than the forward reaction rate at which said compound bonds to said side chain of
10 said free amino acid under physiological conditions.

44. The method of claim 42, wherein said compound bonds to said target protein with a forward reaction rate that is at least 10,000 times faster than the forward reaction rate at which said compound bonds to said side chain of said free amino acid under physiological conditions.

15 45. The method of claim 42, wherein said compound bonds to said target protein with a forward reaction rate that is at least 100,000 times faster than the forward reaction rate at which said compound bonds to said side chain of said free amino acid under physiological conditions.

46. The method of claim 42, wherein said compound has a covalent
20 bonding rate constant with said side chain of said free amino acid of less than 10^{-6} /M/sec at room temperature under physiological conditions.

47. The method of claim 42, wherein said compound has a covalent

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bonding rate constant with said side chain of said free amino acid of less than 10^{-7} /M/sec at room temperature under physiological conditions.

48. The method of claim 42, wherein said target protein is not an enzyme.

5 49. The method of claim 42, wherein said compound is a synthetic compound.

50. The method of claim 42, wherein said protein-protein binding is blocked in a mammal.

10 51. The method of claim 50, wherein said protein-protein binding is blocked in a human.

52. The method of claim 42, wherein said protein-protein binding causes a disease.

53. The method of claim 42, wherein said compound comprises (a) a Specificity Group whose removal results in the bonding reaction rate with said
15 side chain of said free amino acid being substantially unchanged, and the bonding reaction rate with said target protein being reduced to a rate that is substantially similar to the bonding reaction rate with said side chain of said free amino acid; and (b) a Bonding Group that forms the covalent bond with
20 said target protein, wherein modification of said Specificity Group does not substantially alter the bonding reactivity of said Bonding Group.

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54. The method of claim 53, wherein said Bonding Group forms said covalent bond with said side chain of said amino acid on said target protein as a result of the intrinsic reactivity of said Bonding Group.

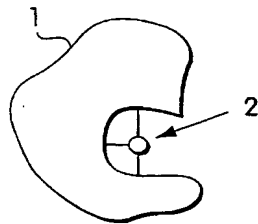


Fig. 1A

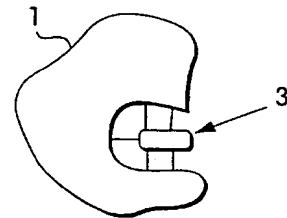


Fig. 1B

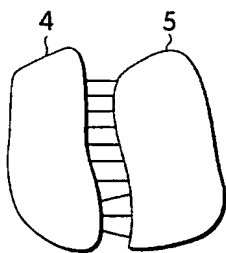


Fig. 1C

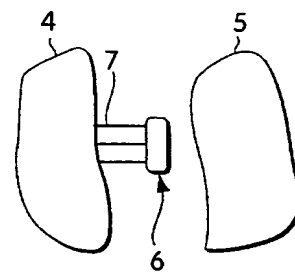


Fig. 1D

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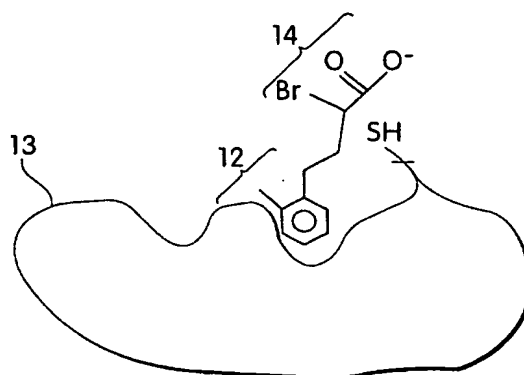


Fig. 2A

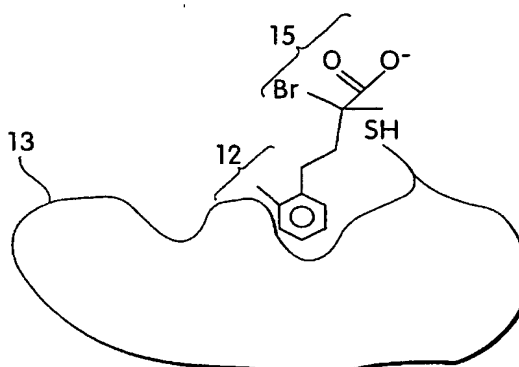


Fig. 2B

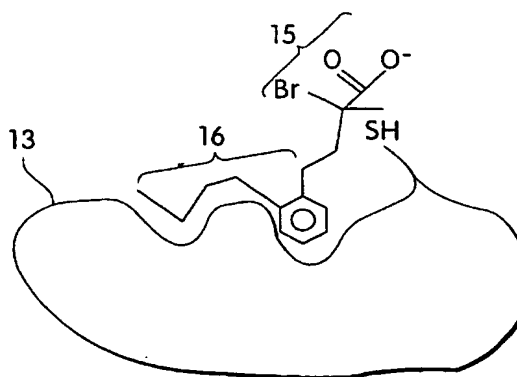


Fig. 2C

SUBSTITUTE SHEET (RULE 26)

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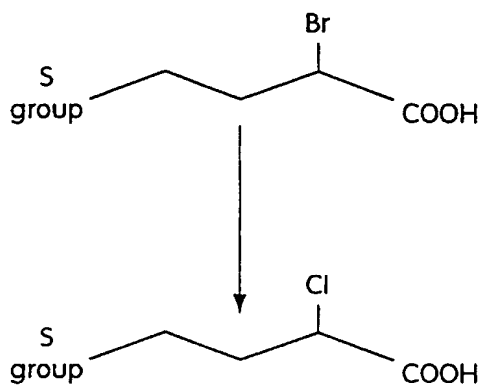


Fig. 3A

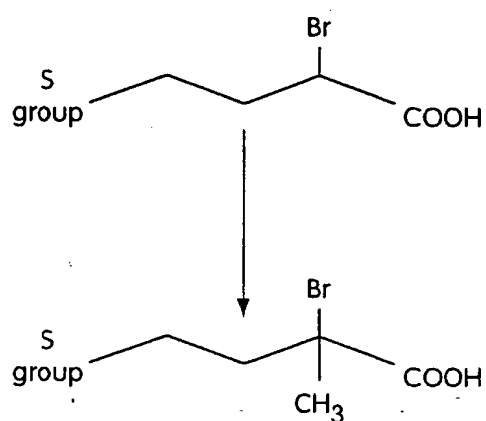


Fig. 3B

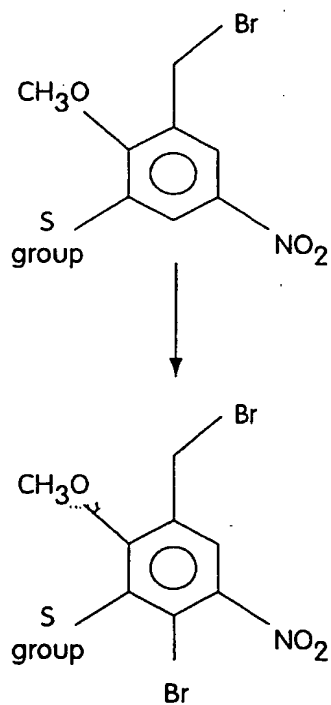


Fig. 3C

SUBSTITUTE SHEET (RULE 26)

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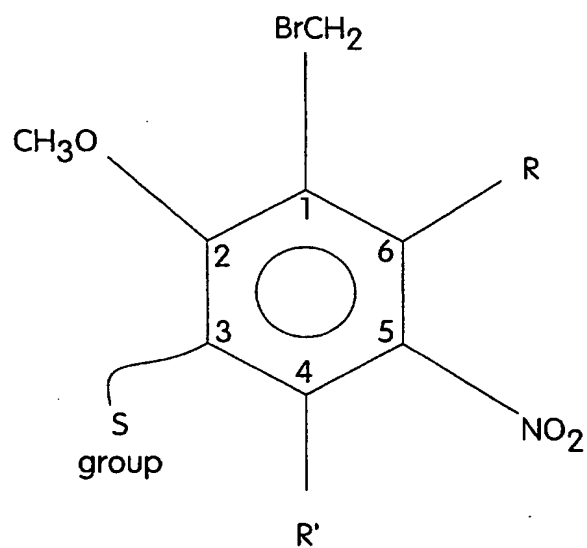


Fig. 4

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25501

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : please see extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 5, 6, 7.1; 436/501, 518

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 5,639,603 A (DOWER et al) 17 June 1997, see entire document especially column 48 lines 19-37, note the use of competitive binding assays to define peptides binding to a protein.	1-46, 48-53 ----- 47, 54
X — Y	BIRNBAUM et al. Peptide Screening. Current Opinion in Biotechnology. 1992, Vol. 3, pages 49-54, see the entire document, especially note the use of competitive ELISA assays in column 2 of page 51.	1-46, 48-53 ----- 47, 54
Y	US 5,571,681 A (JANDA) 05 November 1996, see entire document, especially the abstract. Note that Janda teaches how to find compounds which form covalent linkages from a library of compounds.	47, 54

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

12 MARCH 1999

Date of mailing of the international search report

01 APR 1999

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25501

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 5,658,727 A (BARBAS et al) 19 August 1997, see entire document, especially columns 79 and 80, note the use of competitive binding assays (ELISA) in column 79, line 65 column 80, line 20.	1-46, 48-53 ----- 47, 54

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25501

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C12Q 1/00, 1/70, 1/68; G01N 33/53, 33/566, 33/543

Compounds and compositions are generic and could be in C07; C08; C11 OR C12

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE

2. Where no meaningful search could be carried out, specifically:

Claims 1-32 are directed at compounds and compositions comprising these compounds. The compounds are only limited by generic characteristic and the claims do not recite any structure which would permit a search of the claimed compounds and compositions. Moreover, many of the "functional limitations" recited in the claims do not limit the product because they only recite features that are inherent properties.

In order to proceed with a more compact prosecution the examiner has applied art from the field of peptide chemistry although this in no way reflects the full scope of the claimed invention.